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Grant Title: Study Of Short-Pulsed Laser Retinal Injury Mechanisms By Time-Resolved Imaging Of Photomechanical Transients In RPE

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13. ABSTRACT *(Maximum 200 words)*

We studied RPE cell damage mechanism for laser duration from 100 fsec to 5 usec, and we have investigated the dependence of threshold fluence for cell damage on the laser spot size on the RPE. These results will be summarized in the report. Our current method for studying call damage mechanism in the RPE is stroboscopic time resolved microscopy, which allows us to image the transient microscopic bubble formation inside the RPE tissue ex vivo where the cells following pulse laser irradiation. This technique works well in RPE tissue ex vivo where the strobe light transilluminates the specimen. Since our ultimate goal is to detect the existence of these micobubbles in vivo, we have set up a stroboscopic imaging system in epi-illumination geometry. However, the image quality obtained in this way is inferior to that obtained by transillumination, most likely because of the strong angular dependence of the back-scattered light by the spherical bubbles. It is thus difficult to identify the extent of bubble formation at the RPE by this method.

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Objectives

1. Study laser-induced cavitation bubble formation in RPE cells in vitro using the technique of nsec/psec time-resolved microscopy. Determine the threshold fluence for intracellular cavitation using nanosecond and picosecond laser pulses at 1064 & 532 nm. *(year 1)*

2. Integrate fluorescence imaging capability into existing time-resolved imaging apparatus. Perform RPE cell viability assays and examine the relationship between cell injury and the onset of intracellular cavitation. *(year 1)*

3. Investigate stress wave generation and propagation from target RPE to surrounding cells. Assess the effect of stress transients on neighboring cells not subject to direct laser irradiation and cavitation. *(years 1 and 2)*

4. Investigate the effects of laser spot size on RPE cavitation and cell injury thresholds. *(years 2)*

5. Investigate the possibility of imaging transient bubble formation in RPE in vivo. This will facilitate a direct comparison of in vivo retinal injury threshold with the onset of cavitation. In vivo experiment will be carried out at the Armstrong Laboratory, Brooks AFB. *(year 3)*

6. Investigate the mechanism of multiple pulse laser injury in the RPE. Examine multiple pulse RPE damage using fluences above and below single-pulse mechanical injury threshold to assess the additivity of photomechanical effects. *(year 3)*

7. Investigate femtosecond laser-induced mechanical effects using similar procedures as described above. Femtosecond experiments will be performed using laser sources at Brooks AFB (with the ultrafast laser team in the Armstrong Laboratory). *(year 2)*

Status of Effort

We have studied RPE cell damage mechanism for laser pulse durations from 100 fsec to 5 μsec, and we have investigated the dependence of threshold fluence for cell damage on the laser spot size on the RPE. These results will be summarized in the section below.

Our current method for studying cell damage mechanism in the RPE is stroboscopic time resolved microscopy, which allows us to image the transient microscopic bubble formation inside the RPE cells following pulse laser irradiation. This technique works well in RPE tissue ex vivo where the strobe light transilluminates the specimen. Since our ultimate goal is to detect the existence of these micobubbles in vivo, we have set up a stroboscopic imaging system in epi-illumination geometry. However, the image quality obtained in this way is inferior to that obtained by transillumination, most likely because of the strong angular dependence of the back-scattered light by the spherical bubbles. It is thus difficult to identify the extent of bubble formation at the RPE by this method.

We are currently investigating two other methods to detect bubble formation in vivo - one based on probe beam reflection and one based on photoacoustic detection. We have shown that the probe beam technique is able to detect bubble formation in explant RPE cells very close to threshold. The method is ready to be applied in vivo.

Accomplishments/New Findings

Pulse width dependence studies

The threshold for laser-induced cavitation and cell death in the RPE was determined using a stroboscopic time-resolved microscope ddeveloped for this project. Laser pulse durations of 20 nsec and 30 psec (at 532 nm) were used in the initial experiments and the cell damage threshold was found to be the same as bubble formation threshold. A similar microscope system was assembled at Brooks AFB and the measurements were extended down to 100 fsec. Another system was assembled in the Medical Laser Center in Luebeck and used to probe longer pulse durations (nsec to μsec). The dependence of RPE cell damage threshold as a function of pulse duration over this entire range (from 10^{-13} to 5×10^{-6} sec) is plotted in Figure 1. The threshold is nearly constant below 100 nsec. The increase in threshold fluence above 100 nsec can be attributed to heat diffusion out of the absorbing melanosome particles.

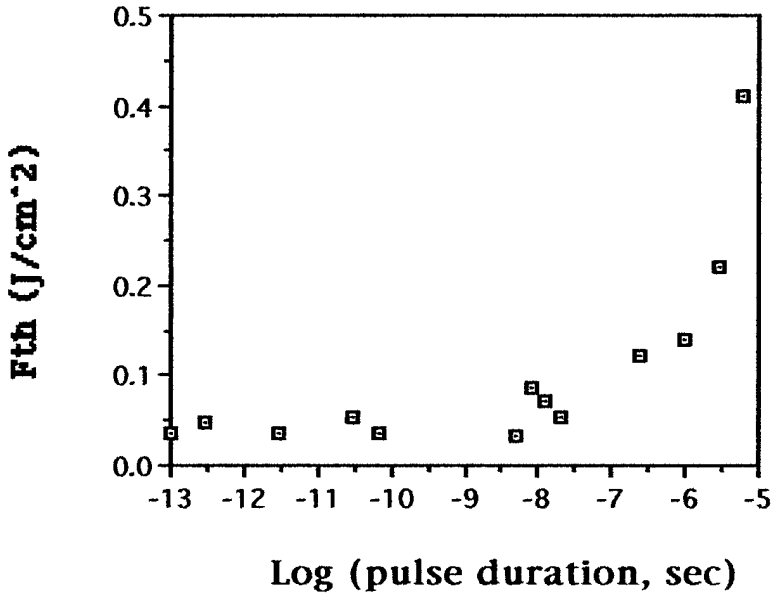

Figure 1. Dependence of RPE cell damage threshold as a function of laser pulse duration. The three data points below 10^{-11} sec were taken with a laser wavelengt of 580 nm. The rest of the data points were taken with 527/532 nm.

Spot size dependence studies

The motivation for the study is the long-standing puzzle from observations made in vivo, that the MVL threshold increases as the retina spot size decreases. For long pulse durations, this effect can be explained by heat diffusion away from the irradiated spot, a process which is more efficient for smaller spot sizes, which in turn requires a higher fluence to cause damage. Surprisingly, the same effect was also observed for short pulse durations where thermal diffusion is not expected to play a significant role, and no satisfactory explanation has been put forth for the observation. At the urging of Dr. David Sliney, we investigated the dependence of threshold for cavitation and cell lethality as a function of laser spot size in the ex vivo model. We expected to find no spot size dependence because the cavitation mechanism requires only a threshold fluence which is independent of spot size. Our results are shown in Figure 2, together with the in vivo data replotted from Beatrice et al. As expected, the ex vivo results show a constant fluence for laser spot sizes ranging from 200 μm to 20 μm (data taken with 100 psec laser pulses at 532 nm). The interesting observation is the absolute laser fluences for producing damage. The in vivo and ex vivo fluence values agree for large spot sizes but diverge significantly for small spot sizes. This raises the question whether a lower fluence exposure in vivo indeed creates damage at the RPE which is not observable ophthalmoscopically because the spot size (and consequently the lesion) is

too small. This question can be answered by probing bubble formation directly in the eye in vivo using the probe beam setup described in the next section.

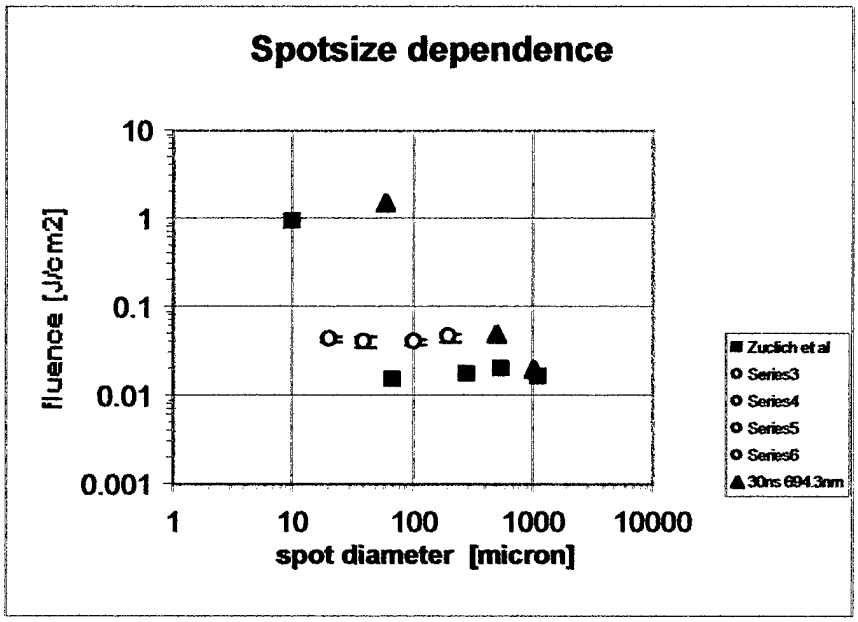

Figure 3. Open circles: ED_{50} threshold for ex vivo RPE cell damage (100 psec, 532 nm).

Filled squares: MVL threshold in vivo, from Zuclich et al.

Filled triangles: MVL threshold in vivo, from Beatrice et al.

Bubble detection by probe beam reflectance

A pump-probe system was set up to detect transient light scattering from the cavitation bubbles, using a diode laser source, confocal detection geometry, and an avalanche photodiode. The diode laser source (830 nm output) is chosen to minimize heating of the RPE cells by the probe beam itself (melanosome absorption decreases toward long wavelengths). In addition, the power of the diode laser is modulated such that the output is effectively turned off during the time between laser pulses in a multiple pulse train. The confocal detection geometry is used to spatially filter out back reflectance and scattering from the optical system and from tissue layers other than the RPE. The use of avalanche photodiode ensures high sensitivity, to detect microbubbles within a single RPE cell, and fast response time, to capture signal from threshold bubbles during their brief lifetime of approximately 100 nsec. Initial results using this system indicates that we can detect near threshold bubbles from a single RPE cell as well as a single melanosome. Figure 3 shows an example of the probe beam reflectance signal. The diode laser was switched on at -10 μsec and switched off at +8.5 μsec to minimize sample heating. The Nd:YAG laser was fired at time zero, which causes a detectable increase in probe beam back scattering (upward signal between 0 and 2

μsec) from cavitation bubble induced in a single RPE cell. This system is being developed for probing transient bubble formation in vivo and for studying multiple pulse laser-tissue interactions.

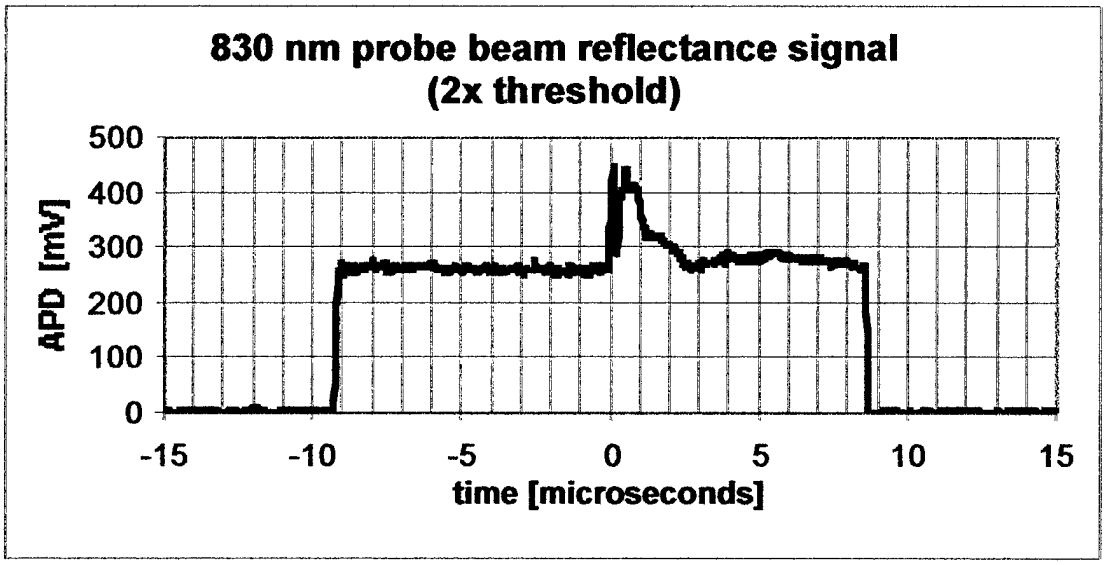

Figure 3. An example of the probe beam reflectance signal from transient cavitation within the RPE. The diode laser was switched on at -10 μsec and switched off at +8.5 μsec to minimize sample heating. The Nd:YAG laser was fired at time zero. Note the upward signal from increased bubble back scattering (between 0 and 2 μsec) which is opposite to the decreased probe transmission signal shown in figure 2.

Personnel Supported

Charles P. Lin	P.I.	25% effort
Jan Roegener	Student	80% effort (10/98-6/99)

Publications

1. Lin CP, Kelly MW, Sibayan SA, Latina MA, Anderson RR. Selective Cell Killing by Microparticle Absorption of Pulsed Laser Radiation. IEEE J Select Topics Quant Electron., 5:963-968 (1999).
2. Brinkmann R, Hüttmann G, Rögener J, Lin CP, Roider J, Birngruber R. Origin of RPE-Cell Damage by pulsed laser irradiance in the ns to μs time regime. Submitted to Lasers Surg Med.
3. Leszczynski D, Pitsillides CM, Anderson RR, Lin CP. Induction of apoptosis and necrosis following pulsed laser irradiation of intracellular pigment microparticles. Submitted to Lasers Surg Med.

Proceedings

1. Hüttmann G, Brinkmann R, Rant B, Birngruber R, Rögener J, Lin CP. "Laser-generated microeffects: microcavitation and absorption properties of melanosomes," in Biomedical Optics: New concepts in therapeutic laser applications, OSA Technical Digest (Optical Society of America, Washington DC, 1999) pp 136-138.
2. Leszczynski D, Pitsillides CM, Anderson RR, Lin CP, "Induction of apoptosis and necrosis following pulsed laser irradiation of intracellular pigment microparticles," in Biomedical Optics: New concepts in therapeutic laser applications, OSA Technical Digest (Optical Society of America, Washington DC, 1999) pp 139-141.
3. Roegener J, Lin CP, "Photomechanical effects - experimental studies of pigment granule absorption, cavitation, and cell damage", Proc Boulder Damage Meeting (1999).

Interactions/Transitions

Results of our RPE studies were presented in three national and international meetings in 1999.

- Optical Society of America topical meeting, Munich, June 1999.
- Boulder Damage Meeting, Boulder, CO, Oct 1999.
- IEEE/LEOS Annual Meeting, San Francisco, CA, Nov 1999.

New discoveries, inventions, or patent disclosures

None.

Honors/Awards

None.